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COULTER COUNTER THEORY AND COINCIDENCE COUNT CORRECTIONS:

MODIFICATIONS AND ADDITIONS FOR WORK WITH SPHERES ABOUT ONE MICRON IN DIAMETER

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UNITED STATES ARMY
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ABSTRACT

This paper deals with the use of the Coulter Counter to count and size particles whose number average volume is between $0.33\mu^3$ and $2.5\mu^3$. It was our purpose to determine whether particles of this size could be accurately counted and sized and to determine whether the existing theory could be applied directly or modified to apply to suspensions of such particles. It has been shown that the implicit assumption of negligible surface conductance of polystyrene particles is acceptable. The device has proved capable of enumerating particles whose volume is at least as small as $0.33\mu^3$, but accurate sizing, employing the volume-threshold relationship developed, is limited to particles whose volume lies between $\sim 0.75\mu^3$ and $2.5\mu^3$. Establishment of empirical curves for each combination of controlled variables will probably permit accurate sizing of particles $0.75\mu^3$ or smaller, the volume range of many commonly studied bacteria.

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I. INTRODUCTION

Early models of the Coulter Counter were designed to count particles in the size-range of erythrocytes; an evaluation of its performance in counting erythrocytes was published by Mattern, Brackett and Olsen.[1] The device has been successfully used for this purpose by a number of other investigators.[2-4] There is general agreement on the approach to the interpretation of the data, insofar as determination of size distribution and count of particles is concerned.

There is no such agreement among workers who have used this counting device on bacterial populations.[5-11] The variety of approaches to interpreting counting and sizing data of these very small particles leads one to question whether the theoretical foundations of the device have been sufficiently developed and whether, in practice, such a device will function effectively with particles smaller than erythrocytes. Kubitschek[11] first modified the Coulter Counter so that it would respond to particles whose diameter is about an order of magnitude smaller than that of erythrocytes. In reporting that work he has shown that an electronic counter operating on the principles employed in the commercially available counter does respond quantitatively to such particles.

The work described in this paper was done to determine what alterations must be made to the present theory of the Coulter Counter[12] in order that the commercially available device may be successfully used to investigate suspensions of particles whose individual volumes are less than that of an erythrocyte. All particles used in this work had number average volumes between $0.33\mu^3$ and $2.5\mu^3$ (number average diameters 0.859μ and 1.68μ).

Assumptions made in deriving the equations of this theory must be examined for their validity when dealing with these smaller particles. Two implicit assumptions, especially, need attention. Coulter[12] has implicitly assumed that surface conductance of particles is negligible. He has also implicitly assumed that calibration curves of threshold setting as a function of volume pass through the origin.

Mattern, Brackett, and Olsen have applied a coincidence count correction based on the Poisson distribution function, but in practice their method puts greatest weight upon those data that are statistically least reliable. Coincidence correction equations (also based on the Poisson distribution) developed by Wales and Wilson[13] depend on assumptions whose validity for the presently available counters has been questioned.[14,15]

Briefly, the Counter continuously monitors the potential required to maintain a chosen current flow through a minute column of conducting liquid, usually physiological saline solution, and responds quantitatively to the increase in potential required to maintain the chosen current flow when a poorly conducting (or nonconducting) particle passes through this column fluid, thus increasing the resistance to current flow. The conducting fluid, with particles suspended in it, is induced to flow through an aperture in a mica plate (the column of conducting fluid is that which fills the aperture) by applying a pressure differential to two chambers separated by the plate. As each particle passes through the aperture, a potential increase occurs whose magnitude is proportional to the volume of the particle. The device is equipped with a discriminator that determines the magnitude of the smallest potential difference to which a built-in recording system will respond. By varying the discrimination level systematically and noting the count at each level, a particle size distribution is obtained.

II. THEORY

This section will present extensions in two areas of the theoretical background of counting and sizing particles. It will first present a discussion of the relationship among certain germane parameters of the Counter itself, the size parameters of the particles (assumed spherical) being counted, and the threshold scale of the Counter. There will then be developed a simple and direct means of correcting the total observed count in a sample volume for coincident passage through the aperture of more than one particle.

A. THRESHOLD READING AS A FUNCTION OF VARIOUS PARAMETERS

Coulter[12] has derived the relationship

$$\Delta R = (8\rho_o d^3/3\pi D^4)(1 - \rho_o/\rho) \qquad (1)$$

in which

ΔR = change in aperture resistance

d = diameter of sphere whose volume equals that of a nonconducting particle in the current path

D = aperture diameter

ρ_o = resistivity of the fluid (generally a solution of an electrolyte) passing through aperture

ρ = resistivity of the particle

In deriving Equation (1) the following assumptions were made:

1) There is an electrically effective volume of electrolyte (critical volume) within which the presence of a particle evokes a response in the associated electrical circuitry; this volume surrounds the ends of the aperture and includes the fluid in the aperture.

2) The magnitude of the critical volume is a simple multiple of that of the aperture volume.

3) The critical volume may be considered to be a right circular cylinder whose circular cross-section diameter equals that of the aperture.

4) Current density through a circular cross section of the critical volume is uniform.

5) The electrically effective volume of a particle within the critical volume may be expressed as the volume of a right circular cylinder whose diameter and length are multiples of a spherical diameter, d, of the particle. The spherical diameter d may or may not correspond to the physical diameter of the particle, but is an electrically effective diameter. The resistivity of the equivalent cylinder is the same as that of the particle and is much greater than that of the suspending fluid.

6) The spherical diameter d is much less than aperture diameter D.

7) Surface conductance of the particle is negligible (implicit assumption).

The threshold dial settings of the Coulter Counter are in direct proportion to the magnitude of the voltage change to which the counting circuit will respond. Therefore we may write

$$T = k\Delta E = kif\Delta R \qquad (2)$$

where

T = threshold dial reading,

ΔE = voltage change required to overcome the increase in resistance due to presence of the particle,

i = current flow through aperture,

k = a proportionality constant dependent on the electronic circuitry,

f = current amplification factor.

In order to keep ΔE within the limited range required by the electronic circuitry when ΔR may vary considerably from one application to another it is necessary to provide a choice of aperture currents and a variety of amplifications.

There are in practice two restrictions on the use of Equation (2) to represent the response of the Coulter Counter to the passage of particles through the aperture. Both of these restrictions arise in the process of matching the fiducial zero on the threshold dial with zero pulse amplitude. First, the matching procedure is carried out with the electrodes shorted out of the circuit. When the circuit is again arranged to include the path between electrodes, through the electrolyte in the aperture, a constant resistance increment (no particles present) is thrown into the circuit. Second, with the electrodes shorted out, the process of matching the fiducial zero on the threshold dial to zero pulse height requires subjective judgment on the part of the operator (a potentiometer within the counter must be adjusted until the counting rate is judged to be at a minimum). An error in judging the internal potentiometer setting is equivalent to moving the threshold dial to some other setting. The effect of these restrictions is to alter the relationship between threshold setting and resistance change due to particle passage to read:

$$T = kif(\Delta R + \Delta R_e) + b, \tag{3}$$

where

ΔR_e = increment in circuit resistance due to inclusion of the electrolyte that is not present when the electrodes are shorted,

b = a constant.

Substituting for both ΔR and ΔR_e from Equation (1) and, assuming spherical particles, substituting $(6V)/\pi$ for d^3,

$$T = (16\rho_o kif/\pi^2 D^4)(1 - \rho_o/\rho)\ (V + V_{eq}) + b, \tag{4}$$

where V_{eq} is the particle-volume equivalent of electrolyte in the circuit.

The conductivity of polystyrene is negligible.[18] Therefore it will be assumed that $(\rho_o/\rho) \ll 1$ and Equation (4) will be rewritten

$$T = (16\rho_o kif/\pi^2 D^4)(V + V_{eq}) + b, \tag{5}$$

B. DETERMINATION OF COINCIDENT PASSAGE CORRECTION

If the passage of a measured volume (sample) of particle-bearing fluid through the aperture is viewed as the passage of a succession of critical volumes whose average particle population is small, the frequency of passage of critical volumes containing zero, one, two, . . . particles will follow the Poisson distribution. It is assumed that all pulses from coincident passage of more than one particle add so as to produce a single pulse, without restriction as to relationship between cumulative particle volume in the critical volume and pulse height.

Let m = average number of particles per critical volume. Then

$$m = \frac{\text{True count of sample}}{\text{No. of critical volumes in sample}}$$

$$= \frac{(\text{No. critical volumes})\,(P_1 + 2P_2 + 3P_3 + \ldots + nP_n + \ldots)}{(\text{No. critical volumes})}$$

$$= \Sigma n P_n \qquad n \geq 0, \text{ and integral} \tag{6}$$

where

n = the number of particles in a critical volume,

P_n = the probability of finding n particles in any critical volume,

$= m^n e^{-m}/n!$, $n \geq 0$, and integral.

The following ratio may be introduced:

$$\frac{N_c}{N_t} = \frac{P_1 + P_2 + P_3 + \ldots + P_n + \ldots}{P_1 + 2P_2 + 3P_3 + \ldots + nP_n + \ldots} = \frac{\Sigma P_n}{\Sigma n P_n} = \frac{\Sigma P_n}{m}, \quad n \geq 1 \tag{7}$$

in which N_o is the observed number of recorded pulses produced by a sample at given threshold setting, taken to be the number of particle passages recorded by the counter, and N_t is the true number of particles exceeding the given threshold setting size present in the sample. The restriction on n is necessary because critical volumes containing no particles pass through the Counter without evoking a response.

It is possible also to evaluate m in the following terms:

$$m = N_t V_c / V_s \tag{8}$$

where V_c is the critical volume and V_s is the volume passing through the aperture in a counting interval. As was assumed in deriving Equation (1),

$$V_c = aV_a \tag{9}$$

where V_a is the aperture volume and $\underline{a}$ is a constant. Hence

$$m = aV_a N_t / V_s \tag{10}$$

$$= k_v N_t$$

Putting Equation (10) into Equation (7) gives

$$N_o = (1/k_v)\Sigma P_n, \qquad n \geq 1. \tag{11}$$

By substituting the terms of the Poisson distribution into Equation (11) (with the applied restriction), and simplifying, one arrives at the following expression:

$$N_o = \sum_{n=1} \frac{(-k_v)^{(n-1)}}{n!} N_t^n \tag{12}$$

Equation (12) demonstrates the importance of the ratio of critical volume to sample volume. Since the sample volume will be a constant in any investigation, this is equivalent to demonstrating the importance of the aperture volume and the constant $\underline{a}$ in Equation (9). The count that will be obtained on a given sample is strongly dependent upon these quantities.

It is hypothetically possible to obtain for a suspension a particle count free of coincidence errors by diluting the suspension enough to reduce to very low value the probability of coincident passage. The count so obtained could then be multiplied by the appropriate volume relationship to obtain a true count for any other dilution of the original suspension. Due regard must, in practice, be given to two factors: (i) although the lower count obtained with the more dilute solution is subject to little coincidence error, it is subject to relatively great statistical variance, and (ii) the reverse situation exists when counts are made on more populous suspensions. The serious objection can be raised against this procedure that the entire structure depends upon the least accurate point, statistically, to establish the true count. It is much more desirable to estimate the true count from the statistically more reliable data, or from all the data.

Reversion of the series in Equation (12) gives the expression:

$$N_t = \sum_{n=1} \frac{k_v(n-1)}{n} N_o^n \qquad (13)$$

For each experimental point one may calculate an estimate of the true count that gave rise to that observation. In order to carry out such calculations one must have an estimate of k_v, which, since one can experimentally determine aperture volume, reduces to assuming a value for the constant $\underline{a}$ of Equation (9). The test of the entire procedure is that a plot of the ratio of calculated total count to relative particle concentration against relative particle concentration be a straight line of zero slope when the value of $\underline{a}$ is properly chosen. The proper value of the constant $\underline{a}$ can be established by trial and error. The curve of calculated N_t against N_o is then a coincidence correction curve useful for all counts made with the aperture.

The calculated value N_t should be corrected by subtracting from it the observed "background" count characteristic of the batch of diluting solution used.

III. MATERIALS AND METHODS

The conducting fluid used in these studies was commercial physiological saline. Before use it was passed twice through cellulose membrane filters, the first filter having a pore diameter of 0.45 micron, the second having a pore diameter of 100 millimicrons.

Filter discs were rinsed by passage of about 50 milliliters of the saline solution, the wash being discarded. With the sole exception of microliter pipettes, all glassware coming into contact with the saline or with suspensions made with it were thoroughly rinsed twice with filtered saline (100 mμ filter) before being used.

Monodisperse polystyrene latex suspensions were used for calibrations.* The number average diameter of these spherical particles, and its standard deviation, were determined by the method of Kubitschek.[17] A small volume (about one microliter) of the supplied suspension was mixed with immersion oil and a cover slip applied. Examination under the microscope revealed monolayer arrays of particles in hexagonal closest packing. The procedure required measuring with a calibrated Filar micrometer lengths of numerous rows in such arrays, counting the number of spheres in each row. From these data were computed the mean diameter and the standard deviation of this mean.

In this work, as in Kubitschek's, Cargille's immersion oil, refractive index = 1.5150, was used. Crown brand of immersion oil was imbibed by the latex spheres in sufficient quantities to increase their volume to ~146% of the original. The only evidence available that Cargille's oil was not imbibed to an appreciable extent lies in the observations themselves (see Section IV, Results) when comparison is made with other published data.

Suspensions to be counted or on which size distribution was to be determined were prepared in two steps. Initially, 25 microliters of the Dow product were made up to 100 ml total volume in filtered saline. The requisite volume of this suspension was then further diluted to 100 ml with filtered saline.

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* Our thanks to Dr. J.W. Vanderhoff and Mr. L.J. Lippie of the Dow Chemical Company, Midland, Michigan, for this material.

The Coulter Counter used in this investigation was a Model B, not modified in any way. This model of the counter is equipped with both discriminator (lower threshold) and veto (upper threshold) controls. The discriminator determines the smallest voltage pulse to which the counting circuit will respond. The veto prevents the counter from responding to voltage pulses exceeding a chosen magnitude. When used in the Model A mode, the veto circuit is disabled and the counting circuit responds to all pulses (particle passages) above a chosen magnitude (particle volume). When used in the Model B mode, both lower threshold and upper threshold are operational, and the Counter records all pulses falling in a chosen range, i.e., counts the passage of particles whose volume lies within a chosen range. This range, referred to as a window, can be arbitrarily established at any convenient width (range of pulse magnitudes or range of particle volumes) by appropriate positioning of lower and upper threshold dials.

It is easier and more direct to use the Counter in the Model B mode to determine average particle volume and particle volume distribution. The size range within which pulses were recorded was chosen so that in obtaining a frequency distribution of volumes there were at least six intervals over the volume range of the particles being counted.

Determination of number average particle volume of particles in suspension requires that the threshold scale of the Counter be calibrated with particles of known number average volume.

The threshold reading corresponding to the number average volume characteristic of each calibrating polystyrene latex was calculated by the equation

$$T = (\Sigma n_i t_i)/\Sigma n_i \qquad (14)$$

where T is the threshold value at the number average volume, t_i is the threshold value of the midpoint of the i-th interval, and n_i is the number of recorded pulses in the i-th interval. The number average particle volume for each monodisperse latex was calculated from the number average particle diameter obtained by Kubitschek's method.*

* To make such calculations is to assume equality between the cube of the first moment of particle diameter, $[(\Sigma d_i\, n_i)/N]^3$, and the third moment of particle diameter $[(\Sigma d_i^3\, n_i)/N]$ [see G. Herdan, Small Particle Statistics (Academic Press, Inc., New York, 1960) 2nd ed. p. 32], and thus assumes perfect uniformity of particle size. Moments cannot be obtained from particle diameter determined by Kubitschek's method. Comparison of first moment cubed and third moment (of particle diameter) for Dow Run LS-464-E, using data on frequency distribution of diameter kindly supplied by Mr. Lippie, revealed that the difference between these quantities was an order of magnitude less than the experimental error of volume determination. This should apply to all the polystyrene latex suspensions used here for each of them shows about the same deviation from uniformity as shown by the stated standard deviation.

There is a different calibration curve for each combination of variables in Equation (5).

Experimental evaluation of V_{eq} and b will require two calibration curves, at different (i x f) combinations. These curves will require at least two experimental points each. For the purpose of making calculations from Equation (5) and the calibration plots, it was convenient to combine the constant terms k, ρ_o, and f of this equation into one constant, k', for each f. Values of k' were determined experimentally for f = 2 and f = 4. The average of these was used for k' at f = 8.

Total particle counts were made using the instrument in the Model A mode.

Aperture dimensions were measured with a microscope. The thickness of the mica plate was determined by focusing a phase contrast microscope successively on scratches on the two surfaces of the mica plate and reading the difference on the fine-focus control of the microscope. By focusing on scratches near the aperture ends we attempted to circumvent any distortions of the plate caused by the perforation process. Aperture diameters were determined with a Filar micrometer that had been compared with a stage micrometer under the same magnification. Vertical illumination was employed when measuring diameters.

IV. RESULTS

A count of particles of all sizes present in commercial saline before it is filtered is of the order of tens of thousands when one uses a 30-μ aperture, i.e., when one sets out to count or measure particles whose volume exceeds about 0.14 μ^3. The filtration process used reduced the count to between 500 and 2500 particles (of volumes 0.14 μ^3 or larger) per 0.05 ml of sample.

The number of points at which calibration can be done is limited by the available number of monodisperse polystyrene latexes whose mean volume is below about 3 μ^3. Number average diameters of particles in four monodisperse polystyrene latexes are given in Table 1. A representative example of the differential distribution of counts obtained in the calibration procedure is shown in Figure 1. The count observed in any window is the number of particles (in a 0.05-ml sample) whose individual volumes lie within the boundaries set by the threshold levels.

TABLE 1. APPARENT MEAN DIAMETERS ($\bar{x}$) AND ESTIMATED MEAN DIAMETERS (d) FROM OPTICAL MEASUREMENTS OF MONODISPERSE POLYSTYRENE LATEXES[a]

	Latex Number			
	LS-424-E	LS-067-A	LS-464-E	L5309-17
N, total number of particles	181	103	145	103
R, number of rows	12	7	15	11
$(\bar{x} \pm SE_{\bar{x}})$, μ	0.862 ± 0.005	1.163 ± 0.003	1.294 ± 0.002	1.702 ± 0.004
$\Delta \bar{x}$, overestimate, in percent of $\bar{x}$	0.3	1.14	1.04	1.09
$(d \pm SE_d)$[b], μ	0.859 ± 0.005	1.149 ± 0.011	1.280 ± 0.018	1.683 ± 0.016
Previous measurement,[c] $(d \pm SE_d)$, μ	-	1.143 ± 0.006		-

a. Apparent mean diameter is sum of all row lengths divided by total number of particles. Estimated mean diameter is value after correction for overestimate due to smaller than average particles in the rows.
b. Includes 0.43 per cent standard error (SE) in the calibration of the Filar micrometer.
c. Data of Kubitschek.[17]

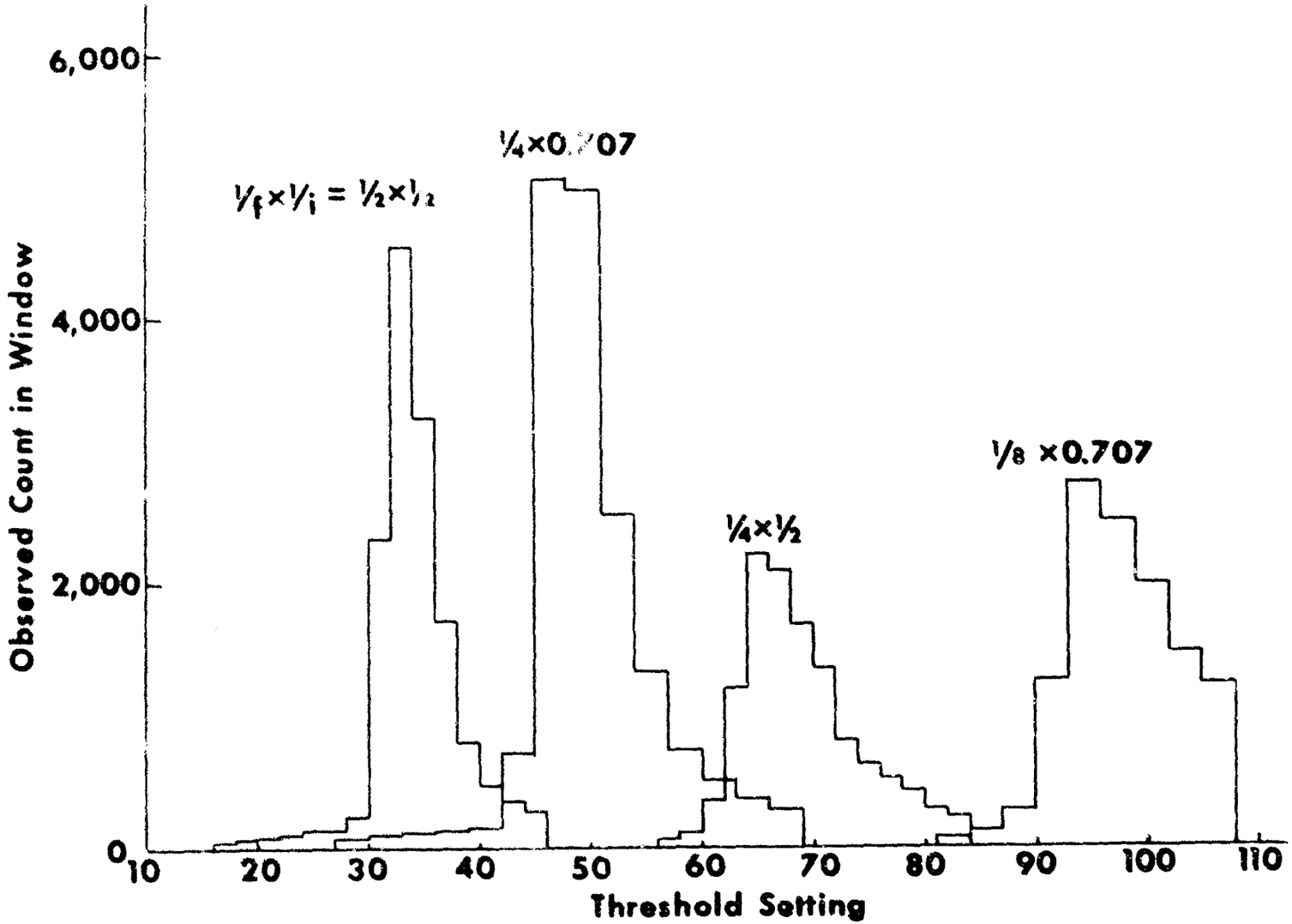

Figure 1. Particle Number Distribution as a Function of Threshold Setting for a Monodisperse Polystrene Latex (Dow Run L5309-17) at Arbitrarily Chosen Combinations of Aperture Current and Amplification Factor. Horizontal line segments show window width by their length, number of pulses counted by their location on vertical axis.

Measurements of aperture dimensions are shown in Table 2, which also includes calculated circular cross-sectional areas and aperture volumes.

Figure 2 shows experimentally determined threshold readings corresponding to the average volume of monodisperse polystyrene latex particles. Two of these curves, labeled A and B, were used to determine the slope of Equation (5) at $f = 2$ and $f = 4$, and provide the basis, along with experimentally determined aperture currents (Table 3), for calculating k', V_{eq}, and b of Equation (5). All other curves in Figure 2 were calculated by Equation (5), using these values of k' and experimental values for aperture current and for aperture diameter.

Coincidence correction curves for selected values of the constant a in Equation (9) are shown in Figure 3. These curves are plots of Equation (13), using aperture volume data from Table 2 and convenient values for observed counts.

Figure 4 shows the plots of (N_t/relative particle concentration) against relative particle concentrations for dilution series prepared from three sizes of Dow polystyrene latex suspensions, assuming in each case three values for a, the constant term in Equation (9).

TABLE 2. MEASURED DIMENSIONS AND CALCULATED QUANTITIES THAT CHARACTERIZE NOMINALLY IDENTICAL APERTURES (30μ)

	Dimensions[a] of Indicated Aperture Number		
	5110	6170	6162
Diameter, μ	33.21(0.36)	36.75(0.52)	37.09(0.13)
Length, μ	28.63(1.30)	24.92(1.27)	26.25(0.30)
Area circular cross-section, μ^2	866.2(13.3)	1060.7(21.2)	1080.4(5.3)
Volume, $10^4\ \mu^3$	2.480(0.166)	2.643(0.210)	2.836(0.052)

a. All averages are based on six or more replicate determinations. Numbers in parentheses are standard deviations.

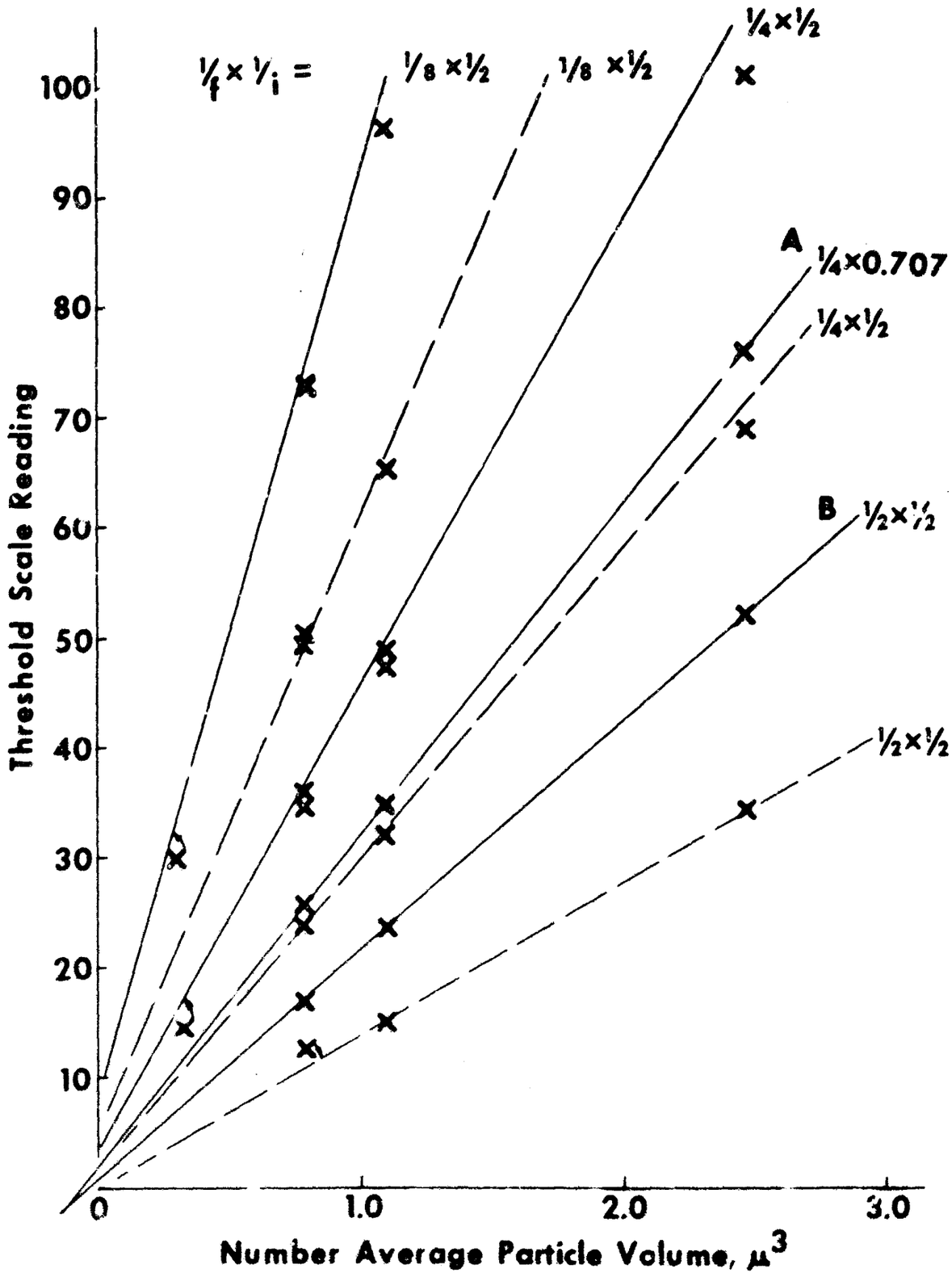

Figure 2. Comparison of Experimental and Calculated Threshold Scale Settings Corresponding to Known Average Volume of Particles. All points shown are experimental. Curves marked A and B were drawn through experimental points using aperture 5110 and provide data for calculating all other lines shown. Curves calculated using Equation (5): solid line, aperture 5110; dashed line, aperture 6170.

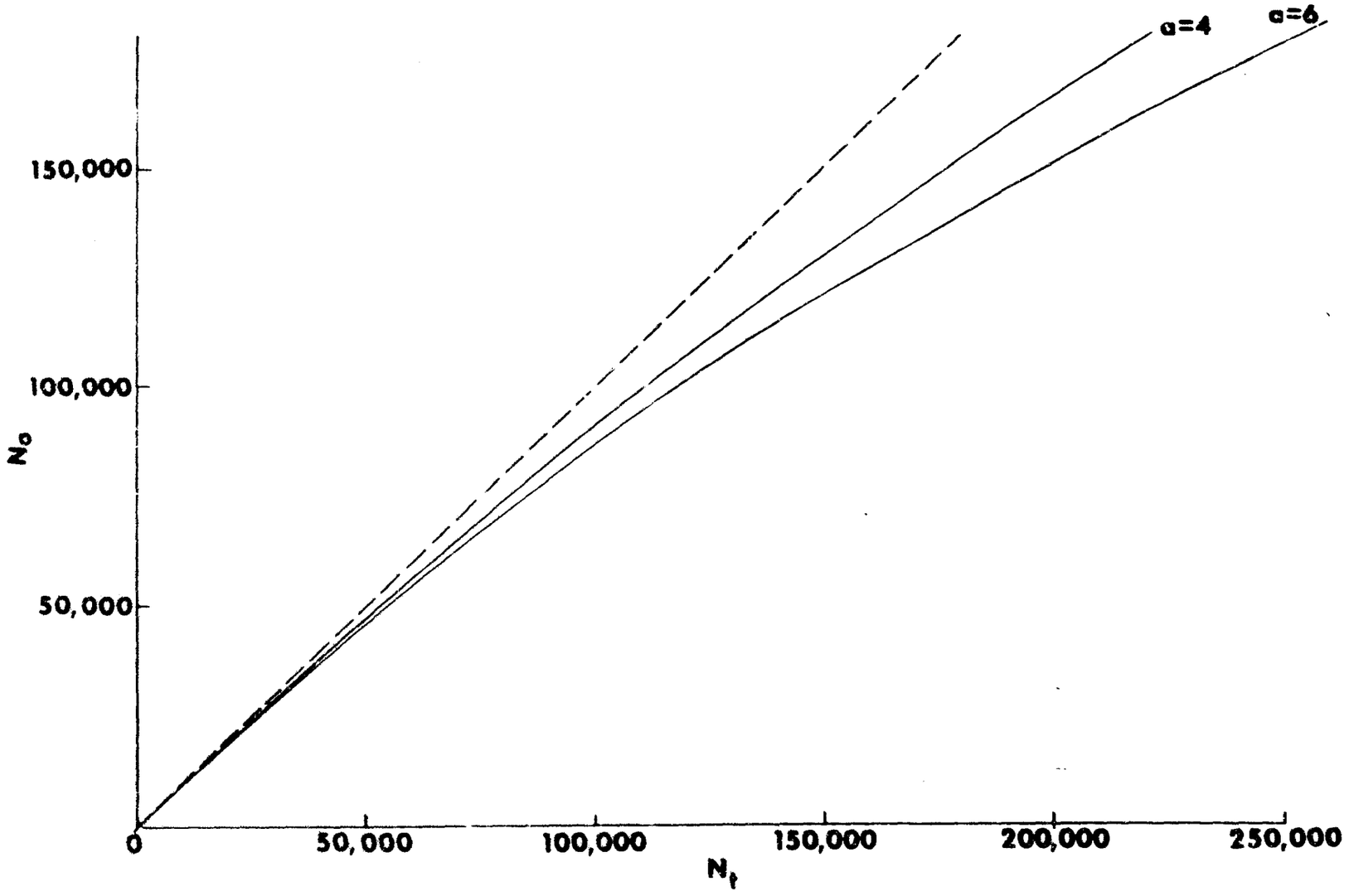

Figure 3. Observed Count as a Function of the Calculated True Count, Showing Decreased Observed Count resulting from Coincident Particle Passage. True count calculated by Equation (13) using selected values of a, the ratio between critical volume and aperture volume. Dashed line: no coincident-passage loss.

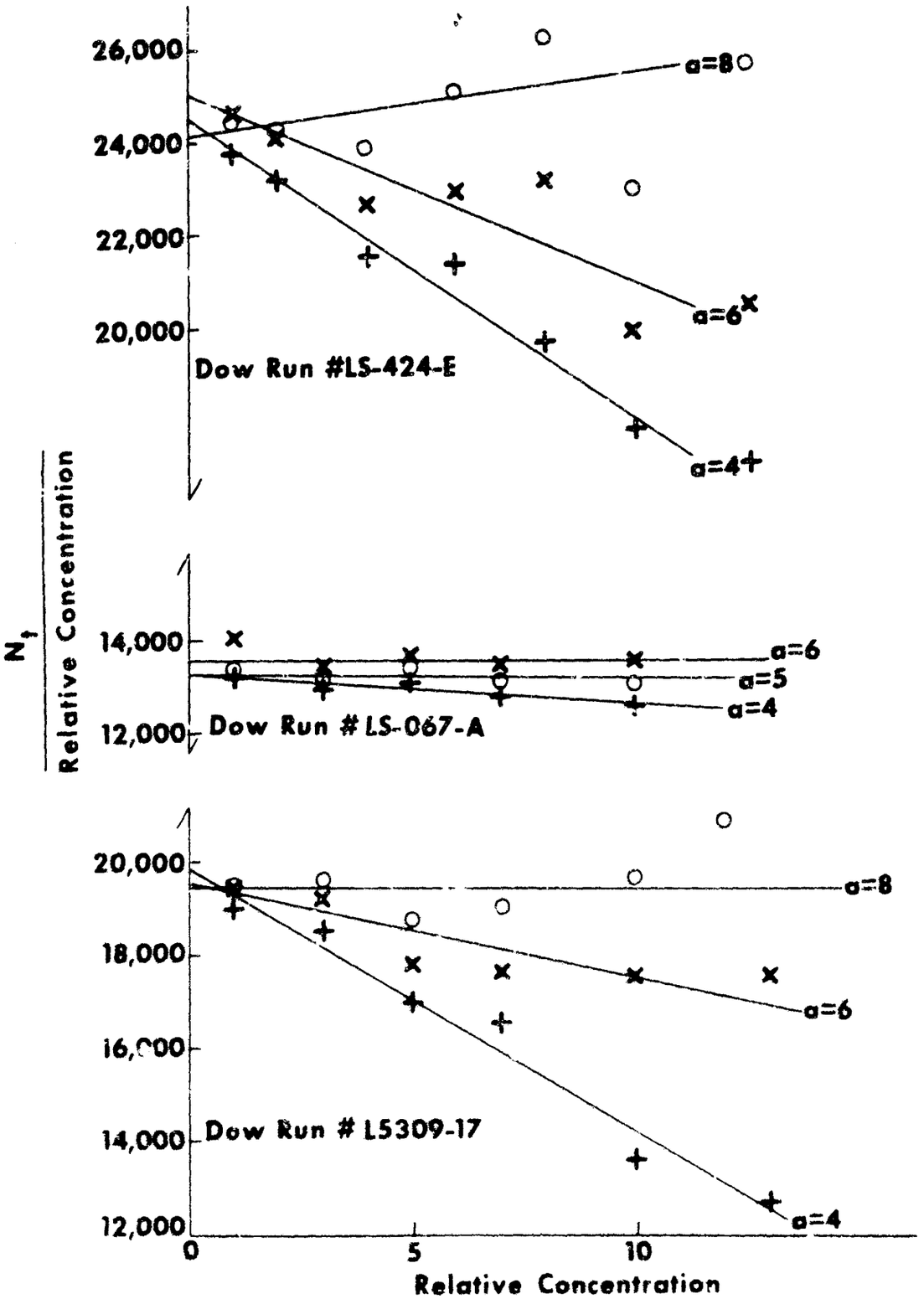

Figure 4. Test of the Procedure for Correcting Observed Count for the Coincident Passage of Two or More Particles. The quantity a is the ratio between critical volume and aperture volume. There exists a value of a that produces a straight line of zero slope in this plot.

TABLE 3. COULTER COUNTER APERTURE CURRENT AT SELECTED SETTINGS OF (RECIPROCAL OF APERTURE CURRENT) SWITCH

	Switch Settings, 1/i		
	1	0.707	1/2
Nominal current flow,[a] μa	500	707	1000
Observed current flow, μa	517	738	1040

a. Data sheet supplied with Counter.

DISCUSSION

The operating procedure for this instrument is one of extreme simplicity, but the accurate assessment of the information it provides requires more attention to certain details than has been given. It is clear from the derivation of Equation (5) that the calibration of this instrument requires more work than the recommended determination of threshold peak corresponding to a single known particle volume.[12] The procedure used here for determining k' may be extended to determine k. To do so would require that f be determined experimentally and that ρ_o be calculated[18] from the electrolyte concentration.

In Figure 1, the distribution of particle number as a function of particle volume is skewed toward larger diameters. This is a consequence of two factors. First, the number distribution of particle diameters is Gaussian.[19] It then follows that the number distribution of particle volumes will be skewed toward greater volume (diameter), producing a volume distribution curve similar to those in this figure. The second factor operating here is the inadequate integration of the voltage pulse produced as a consequence of the short residence time of particles in the aperture.[14,15] This factor results in a falsely high fraction of small pulses, thus tending to skew the volume distribution toward greater volumes.

Figure 2 demonstrates that Equation (5) is capable of representing, within experimental error of measurements, the threshold dependence upon particle volume. This equation also suffices for calculation of response curves of other apertures whose diameter has been determined, once an aperture of measured diameter has been calibrated.

In deriving Equation (5) the assumption numbered 7 (Section II, p. 7) is acceptable if the surface conductance of polystyrene spheres of the size used in this work is of negligible magnitude relative to the conductance of the electrolyte solution displaced. Schwan et al.[16] have stated that the surface layer conductivity of small spheres of polystyrene latex greatly exceeds their specific conductivity; the surface conductance of such particles is usually found to be between 10^{-9} and 10^{-8} mho. The conductance of the displaced physiological saline equivalent-cylinder whose volume equals that of a 1.14μ diameter sphere, being a function of the cylinder's dimensions, can vary from $\sim 2.6 \times 10^{-5}$ mho (cylinder diameter ten times cylinder length) to $\sim 5.5 \times 10^{-8}$ mho (cylinder diameter one-tenth cylinder length).

If the latter model applies, surface conductance is non-negligible; in terms of our equations this would be equivalent to dealing with particles of such low effective resistivity that the quantity (ρ_o/ρ) cannot be neglected and Equation (4) must be applied, rather than Equation (5).

The two experimental points determined using Dow Latex LS-424-E (average volume $0.33\mu^3$) are evidence that there may be limitations on the applicability of Equation (5). This discrepancy cannot be attributed to a surface conductance contribution. If this were the source of difficulty these points would be <u>above</u> the curves drawn using Equation (5), since Equation (4) indicates a numerically smaller slope if the quantity (ρ_o/ρ) must be taken into account. It is concluded that the assumption of negligible surface conductance of the particles is correct.

At present Equation (5) must be considered adequate only when applied to suspensions of particles whose individual volumes lie between $\sim 0.75\mu^3$ and $\sim 2.50\mu^3$. The shape of the function relating threshold to particle volume for particles smaller than $\sim 0.75\mu^3$ must be considered an open question at the moment.

It is necessary to distinguish between the ability of the Coulter Counter to respond to the passage through its aperture of particles smaller than $\sim 0.75\mu^3$ in volume and the ability of the Counter to provide, without further investigation of its properties, an estimate of the volume distribution of such particles. The Counter may be used to determine for a given suspension the number-concentration of particles whose volumes exceed a fixed (but not necessarily known) value at least down to particle volumes of $0.33\mu^3$. If the range of particle-volumes present lies within the range $\sim 0.75\mu^3$ to $\sim 2.50\mu^3$ it is possible to obtain reliable size-distribution data with a Counter calibrated as described in this paper. Accurate sizing of particles whose volume is less than $\sim 0.75\mu^3$ requires experimental calibration of each aperture with particles of known volume. This is equally true when one uses the Counter to produce a cumulative distribution curve (recording passages of all particles exceeding a fixed size and raising the lower threshold in a series of steps) or to produce a differential distribution curve (recording passage of particles whose volume lies between limits set by lower and upper thresholds, and covering a series of impinging windows).

Table 4 shows volume ranges for several frequently studied bacteria, calculated from dimensions given in Bergey's Manual of Determinative Bacteriology.[20] It is clear that one must proceed with caution in attempting to determine volumes of bacteria by this technique, although counts of populations are more often feasible.

Apart from the greater convenience of the electronic counting of bacteria compared with using the Petroff-Hauser Counting Chamber under a microscope, the statistical variation obtained by counting large numbers is much smaller than that obtained by conventional methods. Coulter[12] states that if coincidence correction is held to ~15%, the over-all accuracy of population estimation is better than 1%. Several workers[1, 3, 6] have given different specific counts per unit time or per sample that they state, should not be exceeded if coincidence error is to be kept at or below a specified percentage. The theoretical development presented in this paper shows that there is greater latitude in population density range than was formerly believed. The coincidence error is a function of m, the average number of particles in a critical volume. The error, then is dependent on aperture volume, sample volume, total count present [Equation (8)], and on the constant a [Equation (9)]. Mattern, Brackett, and Olson,[1] counting erythrocytes with an aperture 100μ in diameter and 75μ long, found 15% coincidence error (i.e., observed count was 85% of estimated true count) when the recorded count in a 0.5-ml sample was 60,000. Using Equations (7) and (8) of this paper, and the data from Mattern et al.,[1] one may calculate a true count at which a 15% coincidence error will be observed when counting particles with another aperture. The resulting figure for aperture #5110 (Table 2) is 142,500 particles in 0.05 ml. From Figure 3, such a true count will give an observed count of 124,000. The estimated observed count is 87% of the true count.

Such a counting rate is well within the limitations of the electronic circuitry. In the bank of decade counter tubes two types of counter tubes are used. The first in the series is capable of responding to 10^5 pulses per second.[21] The second tube, and all others, are capable of responding to 4×10^3 pulses per second.[21] The limit of capability of the bank of counters is established by the second tube in the series at 4×10^4 pulses per second at the aperture. Under the pressure differential established across the mica plate of the counter, approximately 15 seconds are required for passage of a 0.05-ml sample. The electronics of the counter thus establish an upper limit of about 6×10^5 particles per sample.

TABLE 4. APPROXIMATE VOLUMES OF FREQUENTLY STUDIED BACTERIA, CALCULATED FROM DATA OF BREED ET AL.[20]

Organism	Shape	Range of Dimensions, μ	Calculated Volume Range, μ^3
Escherichia coli	rods	0.5 x 1.0 - 3.0	0.17 - 0.57
Aerobacter aerogenes	rods	0.5 - 0.8 x 1.0 - 2.0	0.16 - 0.87
Klebsiella pneumoniae	rods	0.3 - 0.5 x 5.0	0.34 - 0.96
Neisseria catarrhalis	spheres	0.6 - 0.8	0.11 - 0.27
Pseudomonas aeruginosa	rods	0.5 - 0.6 x 1.5	0.26 - 0.36
Serratia marcescens	rods	0.5 x 0.5 - 1.0	0.06 - 0.16

The observation is here reported that unfiltered commercial physiological (injection) saline solution contains many thousands of particles per 0.05 ml in the volume range of latex particles used in this study. Although passage of liquids through a cellulose filter (0.45μ pore diameter) is considered sufficient to remove almost all varieties of nonviral living organisms,[22] it was observed that this was not sufficient to remove particles that interfered with the work reported here, hence the procedure adopted. Toennies et al.[9] also found it necessary to pass diluent fluid through a cellulose filter with a 100-mμ pore diameter. Because it was necessary to dilute stock suspensions of polystyrene latex by a factor of at least 40,000 with filtered saline before making counts on it, any non-polystyrene particles present as contaminants were diluted to a point where they were completely innocuous.

These observations indicate the necessity for extreme caution in attempting to use the Coulter Counter to examine fluids such as urine for bacteria;[10] the technique is probably useless for such purposes unless it is suspected that the bacterial count is sufficiently high that one may dilute the urine to a degree that reduces to relatively low levels the population density of naturally occurring crystalloids and cell debris. The bacterial cell volume of the species whose presence is suspected must also be taken into consideration.

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13 ABSTRACT

This paper deals with the use of the Coulter Counter to count and size particles whose number average volume is between $0.33\mu^3$ and $2.5\mu^3$. It was our purpose to determine whether particles of this size could be accurately counted and sized and to determine whether the existing theory could be applied directly or modified to apply to suspensions of such particles. It has been shown that the implicit assumption of negligible surface conductance of polystyrene particles is acceptable. The device has proved capable of enumerating particles whose volume is at least as small as $0.33\mu^3$, but accurate sizing, employing the volume-threshold relationship developed, is limited to particles whose volume lies between $\sim 0.75\mu^3$ and $2.5\mu^3$. Establishment of empirical curves for each combination of controlled variables will probably permit accurate sizing of particles smaller than $0.75\mu^3$, the volume of many commonly studied bacteria.

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